Sequence Alignment 1

PT inserts.
XX
PS Claim 1; SEQ ID NO 2; 56pp; English.
XX
CC The present sequence is the protein sequence for a novel human organic
CC solute transporter, termed hOst1. The invention provides nucleic acid and
CC amino acid sequences for novel organic solute transporters hOst1, hOst2,
CC hOst3 and hOst4 ADO07223-ADO07230. It also provides methods of using the
CC transporters to screen agents, conjugates or conjugate moieties, linked
CC or linkable to agents, for capacity to be transported as substrates
CC through the transporters, and methods of treatment involving oral or
CC intravenous delivery of agents (e.g. drugs) that either alone, or as a
CC result of linkage to a conjugate moiety, are substrates of one of the
CC transporters.
XX
SQ Sequence 340 AA;

Query Match 100.0%; Score 1764; DB 8; Length 340;
Best Local Similarity 100.0%; Pred. No. 6.8e-183;
Matches 340; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 MEPGRTQIKLDPRYTADLLEVLKTNYGIPSACFSQPPTAAQLLRALGPVELALTSILTLL 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MEPGRTQIKLDPRYTADLLEVLKTNYGIPSACFSQPPTAAQLLRALGPVELALTSILTLL 60

Qy         61 ALGSIAIFLEDAVYLYKNTLCPIKRRTLLWKSSAPTVVSVLCCFGLWIPRSLVLVEMTIT 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 ALGSIAIFLEDAVYLYKNTLCPIKRRTLLWKSSAPTVVSVLCCFGLWIPRSLVLVEMTIT 120

Qy        121 SFYAVCFYLLMLVMVEGFGGKEAVLRTLRDTPMMVHTGPCCCCCPCCPRLLLTRKKLQLL 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SFYAVCFYLLMLVMVEGFGGKEAVLRTLRDTPMMVHTGPCCCCCPCCPRLLLTRKKLQLL 180

Qy        181 MLGPFQYAFLKITLTLVGLFLVPDGIYDPADISEGSTALWINTFLGVSTLLALWTLGIIS 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 MLGPFQYAFLKITLTLVGLFLVPDGIYDPADISEGSTALWINTFLGVSTLLALWTLGIIS 240

Qy        241 RQARLHLGEQNMGAKFALFQVLLILTALQPSIFSVLANGGQIACSPPYSSKTRSQVMNCH 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 RQARLHLGEQNMGAKFALFQVLLILTALQPSIFSVLANGGQIACSPPYSSKTRSQVMNCH 300

Qy        301 LLILETFLMTVLTRMYYRRKDHKVGYETFSSPDLDLNLKA 340
              |||||||||||||||||||||||||||||||||||||||
Db        301 LLILETFLMTVLTRMYYRRKDHKVGYETFSSPDLDLNLKA 340
```

RESULT 2
AAE06588
ID AAE06588 standard; protein; 340 AA.
XX
AC AAE06588;
XX
DT 25-SEP-2001 (first entry)
XX
DE Human protein having hydrophobic domain, HP10778.
XX
KW Human; hydrophobic domain; gene therapy; nutritional supplement;
KW cell proliferation; immunomodulatory; autoimmune disorder; antimicrobial;
KW multiple sclerosis; rheumatoid arthritis; insulin-dependent diabetes;
KW haematopoiesis; tissue growth activity; Parkinson's disease; cytostatic;
KW Huntington's disease; Alzheimer's disease; chemotactic; chemokinetic;
KW haemostatic; thrombolytic; tumour growth inhibitor; anabolic;
KW contraceptive; antiinfertility; antiinflammatory.
XX
OS Homo sapiens.
XX
PN WO200149728-A2.
XX
PD 12-JUL-2001.
XX
PF 28-DEC-2000; 2000WO-JP009359.
XX
PR 06-JAN-2000; 2000JP-00000585.
PR 06-JAN-2000; 2000JP-00000588.
PR 11-JAN-2000; 2000JP-00002299.
PR 03-FEB-2000; 2000JP-00026862.
PR 03-MAR-2000; 2000JP-00058367.
XX
PA (PROT-) PROTEGENE INC.
PA (SAGA) SAGAMI CHEM RES CENT.
XX
PI Kato S, Kimura T;
XX
DR WPI; 2001-418355/44.
DR N-PSDB; AAD12583.
XX
PT Human proteins with hydrophobic domains and the nucleic acids encoding
PT them, useful for preventing diagnosing and treating e.g. cancer,
PT Alzheimer's and inflammation.
XX
PS Claim 1; Page 306-308; 563pp; English.
XX
CC The present sequence is human protein with hydrophobic domain, HP10778.
CC The polynucleotide and polypeptide of the invention may be used in the
CC prevention, diagnosis and treatment of diseases associated with
CC inappropriate polypeptide expression. The polynucleotides may be used to
CC produce the polypeptide, by inserting the nucleic acids into a host cell
CC and culturing the cell to express the protein. The polynucleotides and
CC its complementary sequences may also be used as DNA probes in diagnostic
CC assays and also used in gene therapy. The polypeptides may also be used
CC as antigens in the production of antibodies and in assays to identify
CC modulators of polypeptide expression and activity. The polypeptides and
CC nucleic acids may be used as nutritional supplements, to modulate
CC cytokine and cell proliferation activity, to modulate immune stimulation
CC or suppression (e.g. for the treatment of microbial infections and
CC autoimmune disorders such as multiple sclerosis, rheumatoid arthritis and
CC insulin-dependent diabetes), to modulate haematopoiesis, to modulate
CC tissue growth activity (e.g. for the treatment of Parkinson's disease,
CC Huntington's disease and Alzheimer's disease), to modulate activin and
CC inhibin activity (e.g. for controlling fertility), to modulate
CC chemotactic and chemokinetic activity, to modulate haemostatic and
CC thrombolytic activity, to modulate receptor ligand activity, to modulate
CC inflammation and to inhibit tumour growth
XX
SQ Sequence 340 AA;

Query Match 99.9%; Score 1763; DB 4; Length 340;
Best Local Similarity 99.7%; Pred. No. 8.7e-183;
Matches 339; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 MEPGRTQIKLDPRYTADLLEVLKTNYGIPSACFSQPPTAAQLLRALGPVELALTSILTLL 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MEPGRTQIKLDPRYTADLLEVLKTNYGIPSACFSQPPTAAQLLRALGPVELALTSILTLL 60

Qy         61 ALGSIAIFLEDAVYLYKNTLCPIKRRTLLWKSSAPTVVSVLCCFGLWIPRSLVLVEMTIT 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 ALGSIAIFLEDAVYLYKNTLCPIKRRTLLWKSSAPTVVSVLCCFGLWIPRSLVLVEMTIT 120

Qy        121 SFYAVCFYLLMLVMVEGFGGKEAVLRTLRDTPMMVHTGPCCCCCPCCPRLLLTRKKLQLL 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SFYAVCFYLLMLVMVEGFGGKEAVLRTLRDTPMMVHTGPCCCCCPCCPRLLLTRKKLQLL 180

Qy        181 MLGPFQYAFLKITLTLVGLFLVPDGIYDPADISEGSTALWINTFLGVSTLLALWTLGIIS 240
              ||||||||||||||||||||:||||||||||||||||||||||||||||||||||||||||
Db        181 MLGPFQYAFLKITLTLVGLFLIPDGIYDPADISEGSTALWINTFLGVSTLLALWTLGIIS 240

Qy        241 RQARLHLGEQNMGAKFALFQVLLILTALQPSIFSVLANGGQIACSPPYSSKTRSQVMNCH 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 RQARLHLGEQNMGAKFALFQVLLILTALQPSIFSVLANGGQIACSPPYSSKTRSQVMNCH 300

Qy        301 LLILETFLMTVLTRMYYRRKDHKVGYETFSSPDLDLNLKA 340
              |||||||||||||||||||||||||||||||||||||||
Db        301 LLILETFLMTVLTRMYYRRKDHKVGYETFSSPDLDLNLKA 340
```

RESULT 3

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WO 01/49728 A2

(54) Title: HUMAN PROTEINS HAVING HYDROPHOBIC DOMAINS AND DNAs ENCODING THESE PROTEINS

(57) Abstract: The present invention provides human proteins having hydrophobic domains, DNAs encoding these proteins, expression vectors for these DNAs, transformed eukaryotic cells expressing these DNAs and antibodies directed to these proteins.

DESCRIPTION

Human Proteins Having Hydrophobic Domains and DNAs Encoding These Proteins

TECHNICAL FIELD

The present invention relates to human proteins having hydrophobic domains, DNAs encoding these proteins, expression vectors for these DNAs, eukaryotic cells expressing these DNAs and antibodies directed to these proteins. The proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies directed to these proteins. The human cDNAs of the present invention can be utilized as probes for genetic diagnosis and gene sources for gene therapy. Furthermore, the cDNAs can be utilized as gene sources for producing the proteins encoded by these cDNAs in large quantities. Cells into which these genes are introduced to express secretory proteins or membrane proteins in large quantities can be utilized for detection of the corresponding receptors or ligands, screening of novel small molecule pharmaceuticals and the like. The antibodies of the present invention can be utilized for the detection, quantification, purification and the like of the proteins of the present invention.

BACKGROUND ART

Cells secrete many proteins extracellularly. These secretory proteins play important roles in the proliferation control, the differentiation induction, the material transport, the biophylaxis, and the like of the cells. Unlike intracellular proteins, the secretory proteins exert their actions outside the cells. Therefore, they can be administered in the intracorporeal manner such as the injection or the drip, and they possess hidden potentialities as pharmaceuticals. In fact, a number of human secretory proteins such as interferons, interleukins, erythropoietin, thrombolytic agents and the like are currently employed as pharmaceuticals. In addition, secretory proteins other than those described above are undergoing clinical trials for developing their use as pharmaceuticals. It is believed that the human cells produce many unknown secretory proteins. Availability of these secretory proteins as well as genes encoding them is expected to lead to development of novel pharmaceuticals utilizing them.

On the other hand, membrane proteins play important roles, as signal receptors, ion channels, transporters and the like, in the material transport and the signal transduction through the cell membrane. Examples thereof include receptors for various cytokines, ion

channels for the sodium ion, the potassium ion, the chloride ion and the like, transporters for saccharides, amino acids and the like. The genes for many of them have already been cloned. It has been clarified that abnormalities in these membrane proteins are involved in a number of previously cryptogenic diseases. Therefore, discovery of a new membrane protein is expected to lead to elucidation of the causes of many diseases, and isolation of new genes encoding the membrane proteins has been desired.

Heretofore, due to difficulty in the purification from human cells, many of these secretory proteins and membrane proteins have been isolated by genetic approaches. A general method is the so-called expression cloning method, in which a cDNA library is introduced into eukaryotic cells to express cDNAs, and the cells secreting, or expressing on the surface of membrane, the protein having the activity of interest are then screened. However, only genes for proteins with known functions can be cloned by using this method.

In general, a secretory protein or a membrane protein possesses at least one hydrophobic domain within the protein. After synthesis on ribosomes, such domain works as a secretory signal or remains in the phospholipid membrane to be entrapped in the membrane. Accordingly, if the existence of a highly hydrophobic domain is observed in the amino acid sequence of a protein encoded by a cDNA when the

whole base sequence of the full-length cDNA is determined, it is considered that the cDNA encodes a secretory protein or a membrane protein.

OBJECTS OF INVENTION

The main object of the present invention is to provide novel human proteins having hydrophobic domains, DNAs coding for these proteins, expression vectors for these DNAs, transformed eucaryotic cells that are capable of expressing these DNAs and antibodies directed to these proteins.

SUMMARY OF INVENTION

As the result of intensive studies, the present inventors have successfully cloned cDNAs encoding proteins having hydrophobic domains from the human full-length cDNA bank, thereby completing the present invention. Thus, the present invention provides a human protein having hydrophobic domain(s), namely a protein comprising any one of amino acid sequences selected from the group consisting of SEQ ID NOS: 1 to 10, 31 to 40, 61 to 70, 91 to 100 and 121 to 130. Moreover, the present invention provides a DNA encoding said protein, exemplified by a cDNA comprising any one of base sequences selected from the group consisting of SEQ ID NOS: 11 to 30, 41 to 60, 71 to 90, 101 to 120 and 131

to 150, an expression vector that is capable of expressing said DNA by in vitro translation or in eukaryotic cells, a transformed eukaryotic cell that is capable of expressing said DNA and of producing said protein, and an antibody directed to said protein.

This object as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the following description with reference to the accompanying drawings.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03613.

Figure 2: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03700.

Figure 3: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03935.

Figure 4: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10755.

Figure 5: A figure depicting the hydrophobicity/hydrophilicity profile of the protein

encoded by clone HP10760.

Figure 6: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10764.

Figure 7: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10768.

Figure 8: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10769.

Figure 9: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10784.

Figure 10:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10786.

Figure 11:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03727.

Figure 12:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03801.

Figure 13:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03883.

Figure 14: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03913.

Figure 15: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10753.

Figure 16: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10758.

Figure 17: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10771.

Figure 18: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10778.

Figure 19: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10781.

Figure 20:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10785.

Figure 21:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03878.

Figure 22:A figure depicting the

hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03884.

Figure 23:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03934.

Figure 24: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03949.

Figure 25: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03959.

Figure 26: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03983.

Figure 27: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10745.

Figure 28: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10775.

Figure 29: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10782.

Figure 30:A figure depicting the hydrophobicity/hydrophilicity profile of the protein.

Figure 31:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03977.

Figure 32:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10649.

Figure 33:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10779.

Figure 34: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10790.

Figure 35: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10793.

Figure 36: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10794.

Figure 37: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10797.

Figure 38: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10798.

Figure 39: A figure depicting the

hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10800.

Figure 40:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10801.

Figure 41:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03596.

Figure 42:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03882.

Figure 43:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03903.

Figure 44: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03974.

Figure 45: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP03978.

Figure 46: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10735.

Figure 47: A figure depicting the hydrophobicity/hydrophilicity profile of the protein

encoded by clone HP10750.

Figure 48: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10777.

Figure 49: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10780.

Figure 50:A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10795.

DETAILED DESCRIPTION OF THE INVENTION

The proteins of the present invention can be obtained, for example, by a method for isolating proteins from human organs, cell lines or the like, a method for preparing peptides by the chemical synthesis based on the amino acid sequence of the present invention, or a method for producing proteins by the recombinant DNA technology using the DNAs encoding the hydrophobic domains of the present invention. Among these, the method for producing proteins by the recombinant DNA technology is preferably employed. For example, the proteins can be expressed in vitro by preparing an RNA by in vitro transcription from a vector having the cDNA of the present invention, and then carrying out in vitro translation using this RNA as a

template. Alternatively, incorporation of the translated region into a suitable expression vector by the method known in the art may lead to expression of the encoded protein in large quantities in prokaryotic cells such as *Escherichia coli* and *Bacillus subtilis*, or eukaryotic cells such as yeasts, insect cells and mammalian cells.

In the case where the protein of the present invention is produced by expressing the DNA by in vitro translation, the protein of the present invention can be produced in vitro by incorporating the translated region of this cDNA into a vector having an RNA polymerase promoter, and then adding the vector to an in vitro translation system such as a rabbit reticulocyte lysate or a wheat germ extract, which contains an RNA polymerase corresponding to the promoter. The RNA polymerase promoters are exemplified by T7, T3, SP6 and the like. The vectors containing promoters for these RNA polymerases are exemplified by pKA1, pCDM8, pT3/T7 18, pT7/3 19, pBluescript II and the like. Furthermore, the protein of the present invention can be expressed in the secreted form or the form incorporated in the microsome membrane when a canine pancreas microsome or the like is added to the reaction system.

In the case where the protein of the present invention is produced by expressing the DNA in a microorganism such as *Escherichia coli*, a recombinant

expression vector in which the translated region of the cDNA of the present invention is incorporated into an expression vector having an origin which is capable of replicating in the microorganism, a promoter, a ribosome-binding site, a cDNA-cloning site, a terminator and the like is constructed. After transformation of the host cells with this expression vector, the resulting transformant is cultured. Thus, the protein encoded by the cDNA can be produced in large quantities in the microorganism. In this case, a protein fragment containing any translated region can be obtained by adding an initiation codon and a termination codon in front of and behind the selected translated region and expressing the protein. Alternatively, the protein can be expressed as a fusion protein with another protein. Only the portion of the protein encoded by the cDNA can be obtained by cleaving this fusion protein with a suitable protease. The expression vectors for *Escherichia coli* are exemplified by the pUC series, pBluescript II, the pET expression system, the pGEX expression system and the like.

In the case where the protein of the present invention is produced by expressing the DNA in eukaryotic cells, the protein of the present invention can be produced as a secretory protein, or as a membrane protein on the surface of cell membrane, by incorporating the translated region of the cDNA into an expression vector for eukaryotic

cells that has a promoter, a splicing region, a poly(A) addition site and the like, and then introducing the vector into the eukaryotic cells. The expression vectors are exemplified by pKA1, pED6dpc2, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vectors, pRS, pYES2 and the like. Examples of eukaryotic cells to be used in general include mammalian cultured cells such as monkey kidney COS7 cells and Chinese hamster ovary CHO cells, budding yeasts, fission yeasts, silkworm cells, and Xenopus oocytes. Any eukaryotic cells may be used as long as they are capable of expressing the proteins of the present invention. The expression vector can be introduced into the eukaryotic cells by using a method known in the art such as the electroporation method, the calcium phosphate method, the liposome method and the DEAE-dextran method.

After the protein of the present invention is expressed in prokaryotic cells or eukaryotic cells, the protein of interest can be isolated and purified from the culture by a combination of separation procedures known in the art. Examples of the separation procedures include treatment with a denaturing agent such as urea or a detergent, sonication, enzymatic digestion, salting-out or solvent precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing, ion-exchange chromatography, hydrophobic

chromatography, affinity chromatography and reverse phase chromatography.

The proteins of the present invention also include peptide fragments (of 5 amino acid residues or more) containing any partial amino acid sequences in the amino acid sequences represented by SEQ ID NOS: 1 to 10, 31 to 40, 61 to 70, 91 to 100 and 121 to 130. These peptide fragments can be utilized as antigens for preparation of antibodies. Among the proteins of the present invention, those having the signal sequences are secreted in the form of mature proteins after the signal sequences are removed. Therefore, these mature proteins shall come within the scope of the protein of the present invention. The N-terminal amino acid sequences of the mature proteins can be easily determined by using the method for the determination of cleavage site of a signal sequence [JP-A 8-187100]. Furthermore, some membrane proteins undergo the processing on the cell surface to be converted to the secreted forms. Such proteins or peptides in the secreted forms shall also come within the scope of the protein of the present invention. In the case where sugar chain-binding sites are present in the amino acid sequences of the proteins, expression of the proteins in appropriate eukaryotic cells affords the proteins to which sugar chains are added. Accordingly, such proteins or peptides to which sugar chains are added shall also come

within the scope of the protein of the present invention.

The DNAs of the present invention include all the DNAs encoding the above-mentioned proteins. These DNAs can be obtained by using a method for chemical synthesis, a method for cDNA cloning and the like.

The cDNAs of the present invention can be cloned, for example, from cDNA libraries derived from the human cells. The cDNAs are synthesized by using poly(A)$^+$ RNAs extracted from human cells as templates. The human cells may be cells delivered from the human body, for example, by the operation or may be the cultured cells. The cDNAs can be synthesized by using any method such as the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J., Gene 25: 263-269 (1983)] and the like. However, it is desirable to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)], as exemplified in Examples, in order to obtain a full-length clone in an effective manner. In addition, commercially available human cDNA libraries can be utilized. The cDNAs of the present invention can be cloned from the cDNA libraries by synthesizing an oligonucleotide on the basis of base sequences of any portion in the cDNA of the present invention and screening the cDNA libraries using this oligonucleotide as a probe for colony or plaque hybridization according to a method known

in the art. In addition, the cDNA fragments of the present invention can be prepared from an mRNA isolated from human cells by the RT-PCR method in which oligonucleotides which hybridize with both termini of the cDNA fragment of interest are synthesized, which are then used as the primers.

The cDNAs of the present invention are characterized in that they comprise any one of the base sequences represented by SEQ ID NOS: 11 to 20, 41 to 50, 71 to 80, 101 to 110 and 131 to 140 or the base sequences represented by SEQ ID NOS: 21 to 30, 51 to 60, 81 to 90, 111 to 120 and 141 to 150. Table 1 summarizes the clone number (HP number), the cells from which the cDNA clone was obtained, the total number of bases of the cDNA, and the number of the amino acid residues of the encoded protein, for each of the cDNAs.

Table 1

Sequence No.	HP No.	Cell	Number of bases	Number of amino acids
1, 11, 21	HP03613	Kidney	2865	578
2, 12, 22	HP03700	Kidney	3323	243
3, 13, 23	HP03935	Kidney	1585	461
4, 14, 24	HP10755	Kidney	2122	647
5, 15, 25	HP10760	Kidney	1775	446
6, 16, 26	HP10764	Kidney	1372	197
7, 17, 27	HP10768	Kidney	2074	540
8, 18, 28	HP10769	Kidney	2252	442
9, 19, 29	HP10784	Kidney	1461	262
10, 20, 30	HP10786	Kidney	1122	152
31, 41, 51	HP03727	Kidney	1617	335
32, 42, 52	HP03801	Umbilical cord blood	1749	208
33, 43, 53	HP03883	Kidney	1402	406
34, 44, 54	HP03913	Kidney	2474	618
35, 45, 55	HP10753	Umbilical cord blood	3296	208
36, 46, 56	HP10758	Kidney	1818	502
37, 47, 57	HP10771	Kidney	1646	336
38, 48, 58	HP10778	Kidney	1416	340
39, 49, 59	HP10781	Kidney	1927	223
40, 50, 60	HP10785	Kidney	1419	309
61, 71, 81	HP03878	Kidney	2016	599
62, 72, 82	HP03884	Kidney	1446	81
63, 73, 83	HP03934	Kidney	2467	654
64, 74, 84	HP03949	Kidney	1450	390
65, 75, 85	HP03959	Kidney	1897	452

Table 1 (continued)

Sequence No.	HP No.	Cell	Number of bases	Number of amino acids
66, 76, 86	HP03983	Kidney	1856	490
67, 77, 87	HP10745	Umbilical cord blood	2173	392
68, 78, 88	HP10775	Kidney	1934	538
69, 79, 89	HP10782	Kidney	1880	102
70, 80, 90	HP10787	Kidney	2295	442
91, 101, 111	HP03977	Kidney	1894	227
92, 102, 112	HP10649	KB	2413	352
93, 103, 113	HP10779	Kidney	2376	130
94, 104, 114	HP10790	Kidney	1155	330
95, 105, 115	HP10793	Kidney	1329	350
96, 106, 116	HP10794	Kidney	1387	113
97, 107, 117	HP10797	Kidney	1158	189
98, 108, 118	HP10798	Kidney	1106	277
99, 109, 119	HP10800	Kidney	1907	274
100, 110, 120	HP10801	Kidney	1816	390
121, 131, 141	HP03696	Umbilical cord blood	1961	395
122, 132, 142	HP03882	Kidney	2194	550
123, 133, 143	HP03903	Kidney	2753	218
124, 134, 144	HP03974	Kidney	2085	596
125, 135, 145	HP03978	Kidney	2208	467
126, 136, 146	HP10735	Umbilical cord blood	2044	476
127, 137, 147	HP10750	Umbilical cord blood	2176	449
128, 138, 148	HP10777	Kidney	1363	105
129, 139, 149	HP10780	Kidney	1043	81
130, 140, 150	HP10795	Kidney	2435	552

The same clones as the cDNAs of the present

invention can be easily obtained by screening the cDNA libraries constructed from the human cell lines or human tissues utilized in the present invention using an oligonucleotide probe synthesized on the basis of the base sequence of the cDNA provided in any one of SEQ ID NOS: 11 to 30, 41 to 60, 71 to 90, 101 to 120 and 131 to 150.

In general, the polymorphism due to the individual differences is frequently observed in human genes. Accordingly, any cDNA in which one or plural nucleotides are added, deleted and/or substituted with other nucleotides in SEQ ID NOS: 11 to 30, 41 to 60, 71 to 90, 101 to 120 and 131 to 150 shall come within the scope of the present invention.

Similarly, any protein in which one or plural amino acids are added, deleted and/or substituted with other amino acids resulting from the above-mentioned changes shall come within the scope of the present invention, as long as the protein possesses the activity of the protein having any one of the amino acid sequences represented by SEQ ID NOS: 1 to 10, 31 to 40, 61 to 70, 91 to 100 and 121 to 130.

The cDNAs of the present invention also include cDNA fragments (of 10 bp or more) containing any partial base sequence in the base sequences represented by SEQ ID NOS: 11 to 20, 41 to 50, 71 to 80, 101 to 110 and 131 to 140 or in the base sequences represented by SEQ ID NOS: 21 to 30, 51 to 60, 81 to 90, 111 to 120 and 141 to 150. Also, DNA

fragments each consisting of a sense strand and an anti-sense strand shall come within this scope. These DNA fragments can be utilized as the probes for the genetic diagnosis.

The antibody of the present invention can be obtained from a serum after immunizing an animal using the protein of the present invention as an antigen. A peptide that is chemically synthesized based on the amino acid sequence of the present invention and a protein expressed in eukaryotic or prokaryotic cells can be used as an antigen. Alternatively, an antibody can be prepared by introducing the above-mentioned expression vector for eukaryotic cells into the muscle or the skin of an animal by injection or by using a gene gun and then collecting a serum therefrom [JP-A 7-313187]. Animals that can be used include a mouse, a rat, a rabbit, a goat, a chicken and the like. A monoclonal antibody directed to the protein of the present invention can be produced by fusing B cells collected from the spleen of the immunized animal with myelomas to generate hybridomas.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by

administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques;

and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction.

Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or

polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In

Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988;

Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6- Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark,S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun.

11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a

protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable

from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding

costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and

thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II

molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan,

A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will

identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which

will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include; without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby

indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complementary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.